iGEM 2016: Team Pittsburgh
Week 15 Lab Notebook

Monday, August 29

Resuspend thallium hairpin DNAzyme in 588 uL water = 33.19 uM
0.383 g $TlCl_3$ • $4H_2O$ in water = 1M
Cell-free thallium hairpin DNAzyme test

Cleavage reaction from paper: 0.7 uM hairpin
Detection limit = 1.5 nM 10 uM Er
100 nM (Hg interference) or 10 uM Tl

Solution A	2 uL	26 uL
Solution B	1.5 uL	19.5 uL
RNAse inhibitor	0.25 uL x13 reactions =	3.25 uL
Substrate	0.25 uL	3.25 uL
Switch	0.09 uL at 270 ng/uL = 25 ng	1.20 uL
Trigger	0.91 uL	

Triggers:

Condition	
No trigger	0.91 uL water
Hairpin	0.35 uL at 10 uM = 0.7 uM hairpin
Hairpin + Er	0.35 uL at 10 uM = 0.7 uM hairpin 0.5 uL at 100 uM = 10 uM Er 0.51 uL water
Hairpin, Er, 100 nM Tl	0.35 uL at 10 uM = 0.7 uM hairpin 0.5 uL at 100 uM = 10 uM Er 0.5 uL at 1 uM = 100 nM Tl 0.46 uL water
Hairpin, Er, 10 uM Tl	0.35 uL at 10 uM = 0.7 uM hairpin 0.5 uL at 100 uM = 10 uM Er 0.5 uL at 100 uM = 10 uM Tl 0.46 uL water
DNA trigger	0.5 uL at 1 uM = 100 nM 100 nM 0.41 uL water

Should have used Buffer B instead of water

Should have used 0.7 uM DNA trigger, not 100 nM

PROCEDURE:

1. Add 2x what is listed in “Triggers” to separate 1.5 uL Eppendorf tubes
2. Make master mix
3. Add 8.18 uL master mix to each tube from Step 1
4. Spin down tubes
5. Separate each tube into 2 tubes of 5 uL each
6. Incubate 2 hours at 37 degrees

Not much color change (including DNA trigger); leave in incubator

Set incubation to 24 hours, return in 48 hours

Wednesday, August 31

Cell-free lead and thallium test

Solution A	2 uL	34 uL
Solution B	1.5 uL	25.5 uL
RNAse inhibitor	0.25 uL x17 reactions =	4.25 uL
Substrate	0.25 uL	4.25 uL
Switch	0.09 uL at 270 ng/uL = 25 ng	1.53 uL
Trigger	0.91 uL	

Triggers:

Condition	
No trigger	0.91 uL water
Tl Hairpin	0.35 uL at 10 uM = 0.7 uM hairpin 0.56 uL water
Hairpin + Er	0.35 uL at 10 uM = 0.7 uM hairpin 0.5 uL at 100 uM = 10 uM Er 0.51 uL water
Hairpin, Er, 10 uM Tl	0.35 uL at 10 uM = 0.7 uM hairpin 0.5 uL at 100 uM = 10 uM Er 0.5 uL at 100 uM = 10 uM Tl 0.46 uL Buffer B
DNA trigger Tl	0.35 uL at 10 uM 0.56 uL water
Pb Hairpin	0.5 uL at 5 nM = 0.5 nM

	0.41 uL water
Pb Hairpin + lead	0.5 uL at 5 nM = 0.5 nM 0.25 uL Pb at 40 uM = 2 uM Pb 0.15 uL Pb buffer
DNA trigger Pb	0.5 uL at 5 nM = 0.5 nM 0.41 uL water

PROCEDURE:

1. Add 2x what is listed in “Triggers” to separate 1.5 uL Eppendorf tubes
2. Make master mix
3. Add 8.18 uL master mix to each tube from Step 1
4. Spin down tubes
5. Separate each tube into 2 tubes of 5 uL each
6. Incubate 2 hours at 37 degrees

Site-Directed Mutagenesis of LacZ in G switch

PCR with primer sets 1 and 2 (Claire)

Phusion PCR program:

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
30 Cycles	98°C 65°C 72°C	10 seconds 30 seconds 7.5 minutes
Final Extension	72°C	7 minutes
Hold	4°C	